DESIGN, SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING STUDIES AND BIOLOGICAL ACTIVITY OF NOVEL QUINAZOLINONE DERIVATIVES AS POTENTIAL EGFR INHIBITORS

A.Jyothsna[1*], K. Padma Latha[2]

Abstact:
A series of novel quinazolinone derivatives (II-(3a-3j) [(E)-7-chloro-2-(4-((4-substituted benzylidene) amino) phenyl) quinazolin- 4(3H)-one] were synthesised, characterized and biological screened for their in vitro antibacterial, anthelmintic and anticancer activity. All compounds, were synthesized through two steps process and structurally conformed by FTIR, ^{1}HNMR and Mass spectroscopy. Their anticancer activity was assessed using MTT method against MCF-7 and SKOV3 cell lines, the anthelmintic activity was carried out by using Indian Earth warms and the antibacterial activity was carried out by Cup-plate diffusion method. In addition, molecular docking studies was assessed using Autodock Vina. The compound **II-3c** (IC_{50} value of **23.24µg** against MCF-7 and **21.05µg** against SKVO3) exhibited good anticancer activity compared with Doxorubicin as standard. In anthelmintic and antibacterial screening, the compounds, **II-3c, II-3h** and **II-3j** have shown excellent anthelmintic activity and compounds **II-3b, II-3d, II-3f, II-3h** and **II-3j** showed significant antibacterial activity.

Key Words: Quinolone, Anticancer, antibacterial and anthelmintic activities, Molecular Docking, MCF-7 and SKOV3 cell lines.

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1. INTRODUCTION

Design of novel drug-like small molecules based on the pharmacological active scaffolds is a rational and promising direction in modern medicinal chemistry. A series of compounds have been synthesized by the combination of biologically active pharmacophores and utilized medicinal chemists to develop new therapeutics targets with a broad range of biological screenings (1-3). Heterocyclic rings containing nitrogen and sulphur are of much intention as they are therapeutically and pharmacologically more active. These compounds are the building blocks of many pharmaceutical products. Quinazoline-4-one and its derivatives are considerable interest due to their pharmacological properties including anti-cancer [4–6], anti-diabetes [7], antifungal [8], antibacterial [9, 10], antihypertensive [11] and anti-tuberculosis activity [12]. Cancer is a complicated disease due to uncontrolled growth of cells without diferentiation, and an increase in abnormal cells leading to tumor formation [13, 14]. In 2020, one out of every 6 deaths in the world was due to cancer and approximately 10 million people died from cancer that year. Breast, lung, colon, rectum and prostate cancers are the most common cancers worldwide [15]. Chemotherapy, surgery, hormone therapy and radiotherapy are the main cancer treatments based on the stage and type of cancer [16].

Among all heterocyclic moieties, quinazoline-4-one has been taken for this review, as quinazoline-4-one has a very broad spectrum of pharmacological activities with minimum side effects [17]. Quinazoline is a well-known heterocyclic compound having the chemical formula $C_8H_6N_2$ and Quinzoline-4-one having the chemical formula $C_8H_6N_2O$. Quinazoline is a light yellow crystalline solid and is also known as 1,3-diazanaphthalene, which comprises one benzene and one pyrimidine ring.

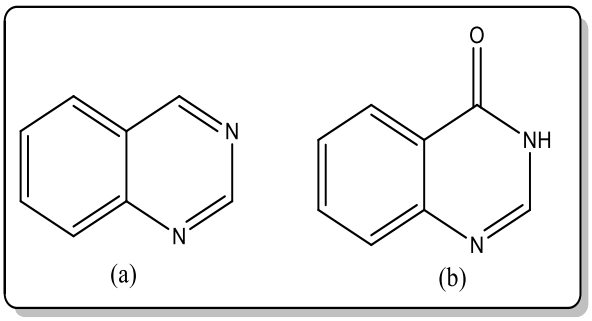


Figure.1. (a) Quinzoline (b) Quinzoline-4-one

Inhibition of epidermal growth factor receptor (EGFR) enzyme is considered to be one of the main mechanisms of quinazolinone analogs as anticancer agents [18]. EGFR is a receptor with tyrosine kinase (TK) activity with significant designation in the cell proliferation, diferentiation, metastasis, and survival cycle [19]. Over activation of EGFR has been reported in some cancerous tissues such as lung, brain, ovarian, colon, breast and prostate tumors [20]. Considering the importance of quinazoline and quinazoline-4-one base structures in cancer treatment, in this study, we synthesized some novel quinazolinone-4-one derivatives II-(3a–3j) to obtain more effective anticancer agents. All the synthesized derivatives were elucidated with ^{1}HNMR, FT-IR and Mass spectroscopy. All compounds were then evaluated against two humans cancerous (MCF-7 and SKOV3) cell lines. In addition, these quinazoline-4-one derivatives were applied to a molecular docking simulation to acquire their binding conformations and structural specificities toward EGFR kinase as plausible targets in cancer treatment.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

Chemicals were used in the synthesis of the final and intermediates were of A.R grade and procured from the Merck and LOBA chemicals. All the synthesized quinazoline-4-one derivatives II(3a-3j) were characterized by melting point determination using Veergo digital melting point apparatus in open capillary tubes and were uncorrected. The IR spectra were recorded using Perkin Elmer FTIR spectrophotometer using KBr pellets techniques, and ^{1}HNMR spectra of the synthesized compounds in deuterated DMSO were recorded on BRUKER AVANCE II 400MHz NMR Spectrometer instrument using TMS as the internal standard. Mass spectra were recorded using LC-MSD-Tranp- SL2010A SHIMADZU using Dimethyl-sulphoxide (DMSO) as a solvent. TLC was performed using silica gel GF254 coated plates of 0.25 mm thickness. Ethyl acetate and n-Hexane (2:8).

2.2. General procedure.

Step: I. Synthesis of (E)-7-chloro-2-(4-amino-phenyl) quinazolin- 4(3H)-one (2a-2b): To a solution of appropriate 4-amino benzaldehyde (1mmol) in ethanol (15 ml), 4-substituted anthranilamide (1 mmol) were added. Make P^H around 4.5 by adding 2-3 drops of glacial acetic acid/adding coper chloride (0.5mmol). The reaction was refluxed for 1-2 hours and the course of reaction was monitored by TLC to its completion. The reaction mixture was cooled by keeping it in room temperature. A solid mass

separated out, which was filtered and washed with water.

Step:I1. Synthesis of (E)-7-chloro-2-(4-((4-substituted-benzylidene) amino) phenyl) quinazolin- 4(3H)-one II-(3a-3j). The compound 7-chloro-2-(4-amino-phenyl) quinazolin- 4(3H)-one (2a-2b) (0.01 mol) was taken in a mixture of substituted benzaldehyde (0.01 mol) and glacial acetic acid (5 mL) and Ethanol 30ml, then the reaction mixture was refluxing for 1-3hrs. The progress of the reaction was monitored by TLC (Hexane: EtoAc 8:2). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off and washed with hexane and recrystallized from methanol to give crystalline solid.

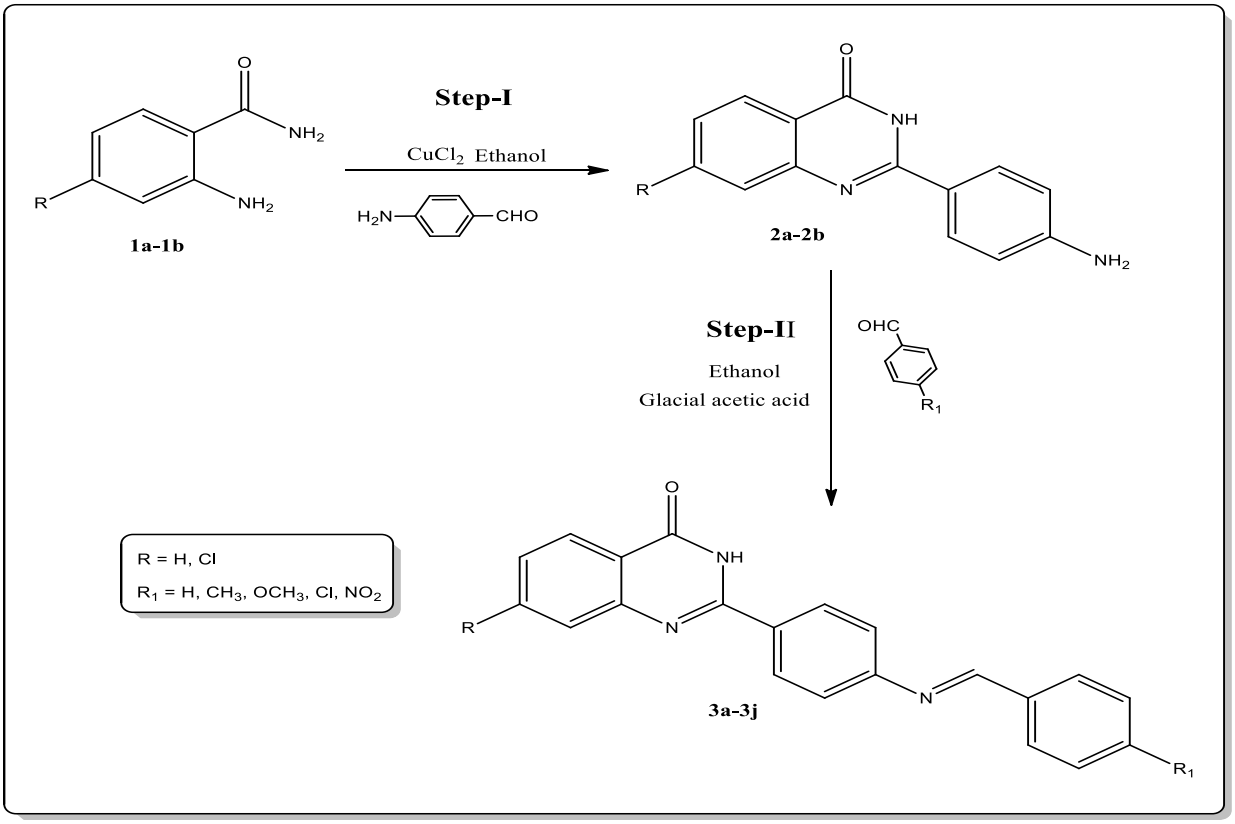


Figure 2. Synthesis scheme for the novel Quinazoline-4-one derivatives II-(3a-3j)

2.3. Biological activity

Antibacterial activity: The synthesized pyrazoline bearing compounds were screened for their in vitro antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive) and *Escherichia coli*, *Salmonella paratyphi*, *Pseudomonas* (Gram negative)) at a concentration of 100 µg/mL by the standard cup plate method [21]. A standard drug (Streptomycin) was used for comparison. Approximately 20-25 mL of freshly prepared liquid agar medium was poured into each petridish and then the petridishes were dried in an incubator at 37 ^{0}C for duration of 1 h. An L-shaped spreader was used to spread the standardized culture of microorganism on each petridish. Cups of approximately 6 mm diameter were made in petridishes using sterile cork borer and were labelled. A solvent control was also tested to see the effect of control on the growth of the microbes. Dimethyl sulfoxide (DMSO) was used to prepare the solutions of synthesized compounds and Streptomycin (100 µg/mL). The prepared solutions were added to each cup in petridishes and were kept aside in an aseptic area for 1 h to allow diffusion of the drug/sample, followed by incubation at 37 ^{0}C for 24 h. The diameter of the zone of inhibition (in mm) was measured and the results are shown in **Table 2**.

Anticancer activity: MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase [22]. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium. Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the

trypan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compound in represented wells in 96 plates.

After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $Ml^{-1)}$ was added to each well and plates were incubated at 37 ^{0} C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The Ic50 value was determined by using linear regression equation i.e. y =mx+c. Here, y = 50, m and c values were derived from the viability graph.

Anthelmintic Activity: The synthesized compounds are screened for anthelminthic activity by using Earth worms [23]. Six earthworms of nearly equal size were placed in standard drug solution and test compound's solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 10 ml with normal saline solution to get the concentration of 0.1% w/v, 0.2 % w/v and 0.5% w/v.

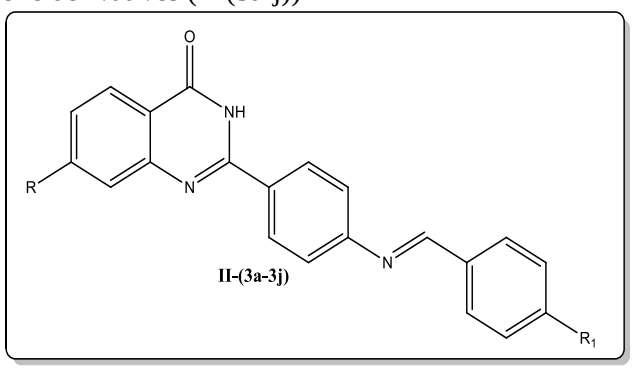
Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms (Figure.5, 6). The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated in **Table No.4.**

Molecular Docking Studies. The 2D structures of 8 compounds were generated from the ACD/Chemsketch Software. The generated ligands cleaned and performed 3D optimization then saved in the MDL Molfile format. The ligands were then converted to a PDBQT file format using the Open Babel chemistry toolbox [24-25]. The three-dimensional (3D) structure of Epidermal Growth Factor Receptor tyrosine kinase (PDB ID: 1M17) was downloaded from Brook Heaven Protein Data Bank (https://www.rcsb.org) and saved as a Brookhaven protein data bank file and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using AUTODOCK suite of MGL Tools. Binding interactions and efficiency of the binding were calculated in terms of dock Score, which is a combination of hydrophilic, hydrophobic, metal binding groups, Vander Waals energy, freezing rotatable bonds and polar interactions with receptor.

3. RESULTS AND DISCUSSION

Synthesis: All novel quinazoline-4-one derivatives II-(3a-3j) were synthesized by a conventional method in two steps. In first step, 4-amino benzaldehyde reacts with 7-substituted anthranilamide vi cyclization reaction in the presence of copper chloride to give 2a-2b compounds. In second step, compound 2a-2b reacts with substituted benzaldehydes in the presence of glacial acetic acid via Schiff's base mechanism to give title derivatives. All the synthesized compounds gave a good yield between 74-88%. Synthesized novel structure were confirmed by FT-IR, ^{1}H NMR and Mass spectroscopy. Finally, all the synthesized compounds were characterized by the physical data **(Table.1)** and spectral properties.

Table. 1. Physical properties novel Quinazoline-4-one derivatives (II-(3a-j))



II-(3a-3j)

S.NO	R	R_1		Mol.For	Mol.Wt	M.P(^{O}C)	%Yield	Rf

II-3a	Cl	H	H	$C_{21}H_{14}ClN_3O$	359.08	121-123	83	0.56
II-3b	Cl	Cl	H	$C_{21}H_{13}Cl_2N_3O$	393.04	143-145	78	0.64
II-3c	Cl	CH_3	H	$C_{22}H_{16}ClN_3O$	373.10	205-207	86	0.78
II-3d	Cl	OCH_3	H	$C_{22}H_{16}ClN_3O_2$	389.09	215-217	76	0.67
II-3e	Cl	NO_2	H	$C_{21}H_{14}ClN_3O$	404.07	233-235	81	0.82
II-3f	Cl	F	H	$C_{21}H_{14}ClN_3O$	377.07	193-195	87	0.77
II-3g	H	Cl	H	$C_{21}H_{14}ClN_3O$	359.08	166-168	76	0.89
II-3h	H	OCH_3	H	$C_{21}H_{14}ClN_3O$	355.13	209-211	69	0.65
II-3i	H	OCH_3	OCH_3	$C_{21}H_{14}ClN_3O$	385.14	151-153	80	0.58
II-3j	Cl	OCH_3	OCH_3	$C_{21}H_{14}ClN_3O$	419.10	183-185	75	0.63

Compound.II-3a: (E)-2-(4-(benzylideneamino) phenyl)-7-chloroquinazolin-4(3H)-one. IR(ν cm^{-1}): 3257(-NH *Str* in quinazolin ring); 3021(-CH Str in Ar); 2919(-CH *Str* in Aliphatic); 1714(-CO Str in quinazolin ring); 1612(-C=N Str); 1425(-C=C *Str* in Ar); 1176(-C-C *Str* in Ar); 823(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 12.1009(s, 1H, -NH proton in quinazolin-4-one ring); 9.7843(s, 1H, Imine proton); 8.4093(s, 1H, Ar-H); 7.8983-7.8694(d, 2H, Ar-H); 7.7909-7.7783(d, 2H, Ar-H); 7.6993-7.6793(d, 2H, Ar-H); 7.5775-7.5673(d, 2H, Ar-H), 7.4984-7.4673(t, 3H, Ar-H). Mass(LC-Ms): m/z 359.08(M); 360.13(M+1, 100%); 361.03(M+2, 30%).

Compound.II-3b: (E)-7-chloro-2-(4-((4-chloro-benzylidene) amino) phenyl)quinazolin-4 (3H)-one. IR(ν cm^{-1}): 3254(-NH *Str* in quinazolin ring); 3012(-CH Str in Ar); 2982(-CH *Str* in Aliphatic); 1701(-CO Str in quinazolin ring); 1633(-C=N Str); 1475(-C=C *Str* in Ar); 1180(-C-C *Str* in Ar); 806(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.8362(s, 1H, -NH proton in quinazolin-4-one ring); 9.6969(s, 1H, Imine proton); 8.0834(s, 1H, Ar-H); 7.8531-7.8054(d, 2H, Ar-H); 7.6864-7.5586(d, 2H, Ar-H); 7.4504-7.4943(d, 2H, Ar-H); 7.3923-7.3784(d, 2H, Ar-H), 7.3428-7.3387(d, 2H, Ar-H). Mass (LC-Ms): m/z 393.04(M); 394.23 (M+1, 100%); 394.21(M+2, 30%).

Compound.II-3c:(E)-7-chloro-2-(4-((4-methylbenzylidene)amino)phenyl)quinazolin-4(3H)-one. IR(ν cm^{-1}): 3224(-NH *Str* in quinazolin ring); 3027(-CH Str in Ar); 2993, 2848(-CH *Str* in Aliphatic); 1716(-CO Str in quinazolin ring); 1615(-C=N Str); 1479(-C=C *Str* in Ar); 1233(-C-C *Str* in Ar); 815(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.5674(s, 1H, -NH proton in quinazolin-4-one ring); 9.3989(s, 1H, Imine proton); 8.1983(s, 1H, Ar-H); 7.8983-7.8674(d, 2H, Ar-H); 7.6984-7.6894(d, 2H, Ar-H); 7.6495-7.6403(d, 2H, Ar-H); 7.5974-7.5843(d, 2H, Ar-H), 7.5675-7.5564(d, 2H, Ar-H); 2.0933(s, 3H, Ar-CH_3). Mass (LC-Ms): m/z 373.10(M); 374.21 (M+1, 100%); 395.32(M+2, 30%).

Compound.II-3d:(E)-7-chloro-2-(4-((4-methoxybenzylidene)amino)phenyl)quinazolin-4(3H) -one. IR(ν cm^{-1}): 3351(-NH *Str* in quinazolin ring); 3016(-CH Str in Ar); 2935, 2813(-CH *Str* in Aliphatic); 1705(-CO Str in quinazolin ring); 1613(-C=N Str); 1427(-C=C *Str* in Ar); 1198(-C-C *Str* in Ar); 791(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 12.2563(s, 1H, -NH proton in quinazolin-4-one ring); 9.8674(s, 1H, Imine proton); 8.4984(s, 1H, Ar-H); 7.9854-7.8543(d, 2H, Ar-H); 7.6998-7.6904(d, 2H, Ar-H); 7.5896 (d, 2H, Ar-H); 7.5786(d, 2H, Ar-H); 7.4634-7.4102(d, 2H, Ar-H), 7.5675-7.5564(d, 2H, Ar-H); 3.6844(s, 3H, Ar-OCH_3). Mass(LC-Ms): m/z 389. 09(M); 390.25(M+1, 100%); 391.41(M+2, 30%).

Compound.II-3e:(E)-7-chloro-2-(4-((4-nitrobenzylidene)amino)phenyl)quinazolin-4(3H)-one. IR(ν cm^{-1}): 3245(-NH *Str* in quinazolin ring); 3028(-CH Str in Ar); 2945(-CH *Str* in Aliphatic); 1703(-CO Str in quinazolin ring); 1621(-NO_2 Str, Ar-NO_2); 1597(-C=N Str); 1403(-C=C *Str* in Ar); 1198(-C-C *Str* in Ar); 800(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.8743(s, 1H, -NH proton in quinazolin-4-one ring); 9.5632(s, 1H, Imine proton); 8.2873(s, 1H, Ar-H); 7.8974-7.8032(d, 2H, Ar-H); 7.7123-7.7032(d, 2H, Ar-H); 7.4763(d, 2H, Ar-H); 7.2663(d, 2H, Ar-H); 7.2983-7.1983(d, 2H, Ar-H), 7.1092-7.0932(d, 2H, Ar-H). Mass(LC-Ms): m/z 389.09 (M); 390.25(M+1, 100%); 391.41(M+2, 30%).

Compound.II-3f:(E)-7-chloro-2-(4-((4-fluorobenzylidene)amino)phenyl)quinazolin-4 (3H)-one. IR(ν cm^{-1}): 3244(-NH *Str* in quinazolin ring); 3065(-CH Str in Ar); 2987(-CH *Str* in Aliphatic); 1716(-CO Str in quinazolin ring); 1621(-C=N Str); 1433(-C=C *Str* in Ar); 1187(-C-C *Str* in Ar); 802(-Cl *Str* in Ar-Cl), 798(-F, Ar-F). ^{1}H-NMR(DMSO) δ ppm: 11.9872(s, 1H, -NH proton in quinazolin-4-one ring); 9.6875(s, 1H, Imine proton); 8.3423(s, 1H, Ar-H); 8.1232-8.0352(d, 2H, Ar-H); 7.8976-7.7652(d, 2H, Ar-H); 7.5432-7.4252(d, 2H, Ar-H); 7.2873-7.2093(d, 2H, Ar-H), 6.9873-6.48754(d, 2H, Ar-H). Mass

(LC-Ms): m/z 377.07(M); 378.21(M+1, 100%); 379.01(M+2, 30%).

Compound .II-3g: (E)-2-(4- ((4-chlorobenzy lidene) amino)phenyl)quinazolin-4(3H)-one. IR(ν cm^{-1}): 3265(-NH *Str* in quinazolin ring); 3023(-CH Str in Ar); 2987(-CH *Str* in Aliphatic); 1712(-CO Str in quinazolin ring); 1621(-C=N Str); 1454(-C=C *Str* in Ar); 1209(-C-C *Str* in Ar); 810(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.8651(s, 1H, -NH proton in quinazolin-4-one ring); 9.8871(s, 1H, Imine proton); 8.3843-8.3675(d, 2H, Ar-H); 7.8989-7.8093(t, 2H, Ar-H); 7.7987-7.7897(d, 2H, Ar-H); 7.7102-7.7005 (d, 2H, Ar-H); 7.5987-7.5544(d, 2H, Ar-H); 7.1364-7.1093(d, 2H, Ar-H). Mass(LC-Ms): m/z 359.12 (M); 360.02(M+1, 100%); 361.09(M+2, 30%).

Compound.II-3h: (E)-2-(4-((4-methoxybenzy lidene) amino)phenyl) quinazolin-4(3H)-one. IR (ν cm^{-1}): 3248(-NH *Str* in quinazolin ring); 3044(-CH Str in Ar); 2965, 2878(-CH *Str* in Aliphatic); 1717(-CO Str in quinazolin ring); 1632(-C=N Str); 1423(-C=C *Str* in Ar); 1212(-C-C *Str* in Ar); 804(-Cl *Str* in Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 11.7643(s, 1H, -NH proton in quinazolin-4-one ring); 9.5632(s, 1H, Imine proton); 8.3774-8.3092(d, 2H, Ar-H); 7.6523-7.5632(t, 2H, Ar-H); 7.4763-7.3763(d, 2H, Ar-H); 7.2091-7.1982(d, 2H, Ar-H); 7.1009-7.0832(d, 2H, Ar-H); 6.9024-6.8722(d, 2H, Ar-H); 3.6854(s, 3H, Ar-OCH_3). Mass(LC-Ms): m/z 355.13(M); 356.03(M+1, 100%); 357.32(M+2, 30%).

Compound.II-3i:(E)-2-(4-((3,4-dimetho xybenzylidene)amino)phenyl)quinazolin-4(3H) -one. IR (ν cm^{-1}): 3254(-NH *Str* in quinazolin ring); 3077(-CH Str in Ar); 2987, 2892(-CH *Str* in Aliphatic); 1702(-CO Str in quinazolin ring); 1619(-C=N Str); 1434(-C=C *Str* in Ar); 1232(-C-C *Str* in Ar). ^{1}H-NMR(DMSO) δ ppm: 12.0934(s, 1H, -NH proton in quinazolin-4-one ring); 9.8201(s, 1H, Imine proton); 8.1763-8.0932(d, 2H, Ar-H); 7.99832(s, 1H, Ar-H); 7.8932-7.7982(d, 2H, Ar-H); 7.6543-7.5673(d, 2H, Ar-H); 7.4873-7.3209(d, 2H, Ar-H); 6.8706-6.7652(t, 2H, Ar-H); 3.7862-3.6843(s, 6H, Ar-OCH_3). Mass (LC-Ms): m/z 385.14(M); 386.34 (M+1, 100%).

Compound.II-3j: (E)-7-chloro-2-(4-((3,4-dimethoxybenzylidene) amino) phenyl) quinazolin-4(3H)-one. IR (ν cm^{-1}): 3265(-NH *Str* in quinazolin ring); 3081(-CH Str in Ar); 2987, 2856(-CH *Str* in Aliphatic); 1710(-CO Str in quinazolin ring); 1623(-C=N Str); 1432(-C=C *Str* in Ar); 1265(-C-C *Str* in Ar), 805(-Cl, Ar-Cl). ^{1}H-NMR(DMSO) δ ppm: 12.3423(s, 1H, -NH proton in quinazolin-4-one ring); 9.6843(s, 1H, Imine proton); 8.3542(s, 1H, Ar-H); 8.1083(s, 1H, Ar-H); 8.1078-8.1002(d, 2H, Ar-H); 7.9872-7.8094(d, 2H, Ar-H); 7.6873-7.5732(d, 2H, Ar-H); 7.2984-7.5463(s, 2H, Ar-H); 3.8092-3.7192(s, 6H, Ar-OCH_3). Mass(LC-Ms): m/z 419.10(M); 420.12 (M+1, 100%), 421.03(M+2, 30%).

Antibacterial activity: Novel Quinazoline-4-one derivatives were screened for antibacterial activity by agar diffusion method. From the results, compounds **II-3b** (23* against *Staphylococcus aureus* and 28* against *Klebsiella pneumonia*); **3II-h**(27*, 25*, 28* against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*); **II-3j** (28* against *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae)* by comparison with standard drug.

Table. 2: Antibacterial activity by zone of inhibition (in mm)- novel quinazoline-4-one derivatives II(3a-3j).

Compound	**Zone of Inhibition (in mm)**			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***
Streptomycin (100µg/ml)	32	30	32	34
II-3a	17	12	10	13
II-3b	16	**26**	14	**28**
II-3c	19	10	09	12
II-3d	20	**25**	**26**	17
II-3e	09	14	13	09
II-3f	**27**	13	**25**	**26**
II-3g	18	13	12	09
II-3h	**27**	**35**	**28**	13
II-3i	17	16	10	13
II-3j	**25**	**25**	14	**28**

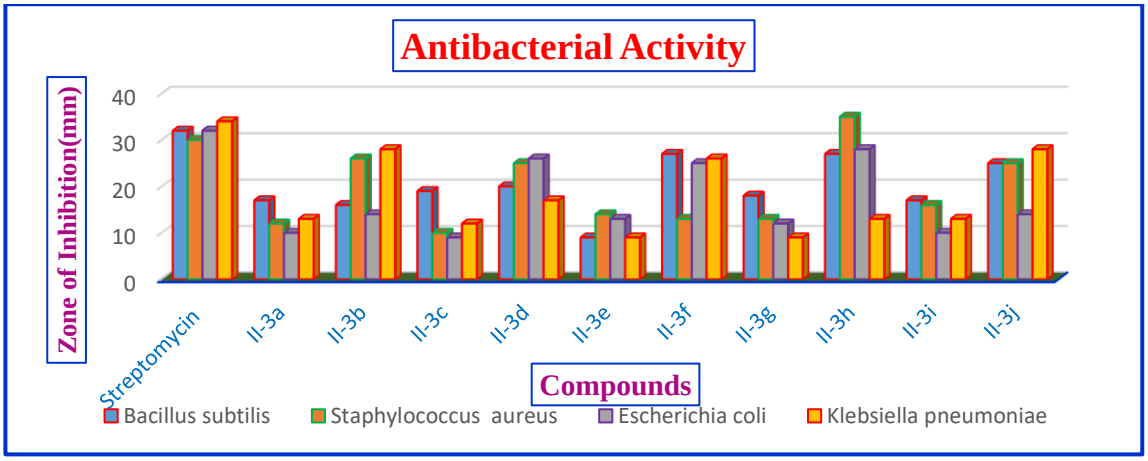


Fig.3: Graphical representation of various novel quinazoline-4-one derivatives II(3a-3j)-Anti-bacterial activity

Fig.4: Photographs of various novel quinazoline-4-one derivatives II(3a-3j)-Anti-bacterial activity

Anticancer activity: The novel Quinazolinone derivatives was tested for their anticancer activity against two cancer cell lines like MCF-7 and SKOV3 by using MTT assay method and doxorubicin as a standard drug. The results of anticancer screening of novel quinazoline-4-one derivatives were expressed as IC_{50} values are summarized in **Table 3**

Table.3. Anticancer activity of novel quinazoline-4-one derivatives II(3a-3j) on MCF-7 and SKOV3 Cell lines.

S. No	SAMPLE NAME	IC_{50} (µg) MCF -7	IC_{50} (µg) SKOV3
1	II-3a	41.07	**16.39**
2	**II-3c**	**23.24**	**21.05**
3	II-3e	43.97	46.57
4	II-3f	52.04	43.84
5	II-3h	54.67	51.24
6	II-3j	25.76	47.43
13	Doxorubicin	12.23	15.23

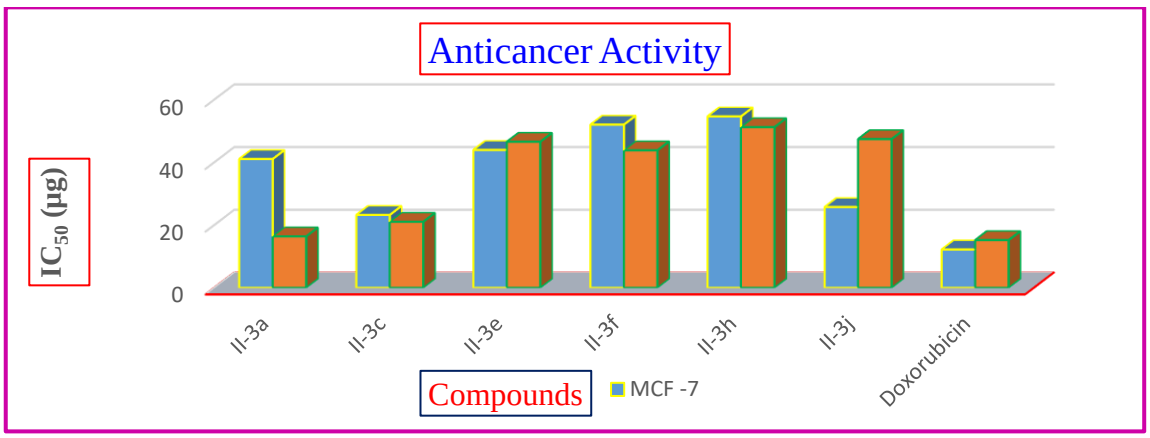


Figure 5: Graphical representation of Anticancer activity of novel quinazoline-4-one derivatives II(3a-3j) on MCF-7 and SKOV3 Cell lines.

From resulting data(Table 2), it is observed that compound the IC_{50} values in the range of 23.24µg to 54.67µg against MCF7 cell line and 16.39µg to 51.24µg against SKOV3 cell line. The compound **II-3c** (IC_{50} value of **23.24µg** against MCF-7 and 21**.05µg** against SKOV3) and compound **II-3a** (IC_{50} value of **16.39µg** against MCF-7 exhibited good anticancer activity compared with Doxorubicin as standard, whereas remaining are moderated activity.

Anthelmintic activity: Novel quinazolin-4-one derivatives **II-(3a-3j)** were evaluated for anthelmintic activity on Indian earthworms (*Pheretima posthuma*). All compounds showed anthelmintic activity is shown in table 4.

Among the compounds tested all the compounds were showed significant paralytic time of earthworms, compared to standard drug albendazole at 0.1%, 0.2% and 0.5% concentrations of compounds. A closer inspiration of data from this table indicated that the synthesized compound **II-3c, II-3h** and **II-3j** showed good Anthelmintic activities whereas others showed significant activities.

Table.No.4. Anthelmintic activity of novel quinazoline-4-one derivatives II(3a-3j)

S.No.	Name	Time in minutes					
		For paralysis % Concentration			For death % Concentration		
	Concentration	0.1	0.2	0.5	0.1	0.2	0.5
	Control	-	-	-	-	-	-
	Albendazole	16	12	8	41	33	25
1	II-3a	29	24	21	56	48	38
2	II-3b	35	29	26	57	48	42
3	II-3c	**17**	**14**	**11**	**48**	**37**	**30**
4	II-3d	34	28	23	63	51	47
5	II-3e	31	24	20	66	54	43
6	II-3f	32	25	19	58	49	43
7	II-3g	30	29	23	61	46	40
8	II-3h	**18**	**15**	**12**	**47**	**38**	**32**
9	II-3i	29	24	20	62	54	45
10	II-3j	**17**	**16**	**13**	**47**	**40**	**33**

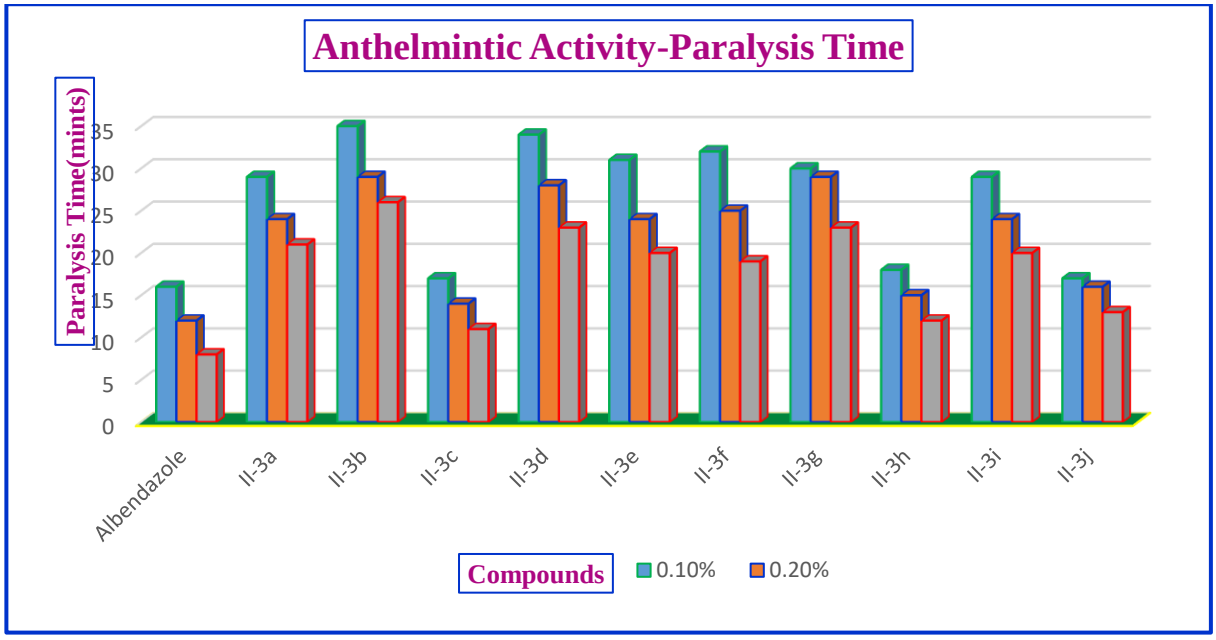


Figure.No.6: Graphical representation of anthelmintic activity of novel quinazoline-4-one derivatives II(3a-3j)– Paralysis time (min).

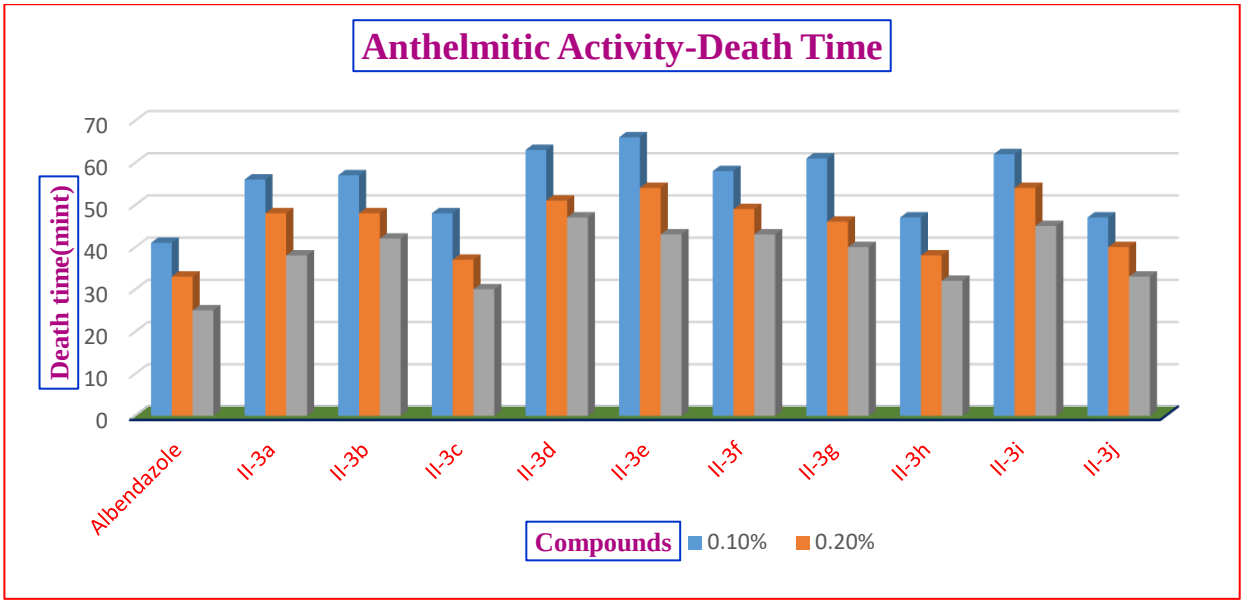


Figure.No.7: Graphical representation of anthelmintic activity of novel quinazoline-4-one derivatives II(3a-3j)-Death time (min).

Fig.4: Photographs of various novel quinazoline-4-one derivatives II(3a-3j)-Anti-bacterial activity

Molecular Docking Studies: Molecular docking studies were performed in order to find the possible protein ligand interactions of the dataset ligands. The potential active site amino acids of 1M17 complex were predicted using CASTp.

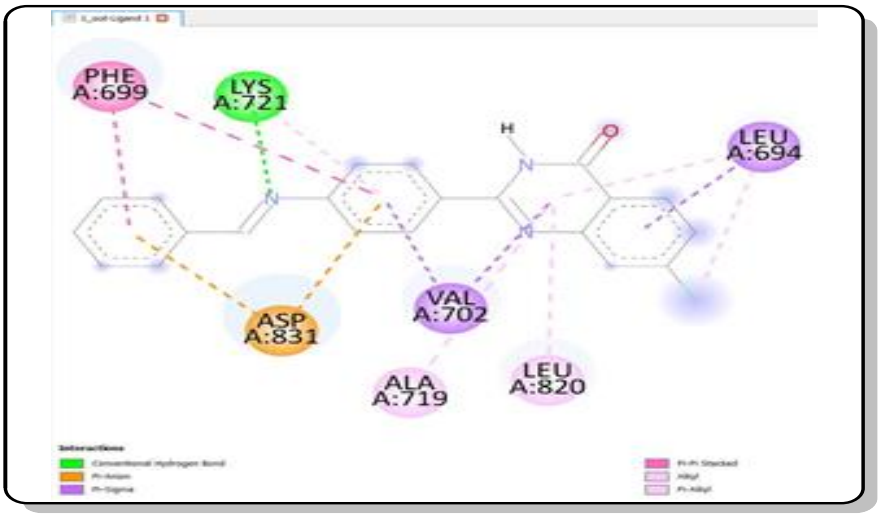
The target protein and inhibitors were geometrically optimized. All the 8 compounds were docked against active site of target protein using AUTODOCK VINA. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About 100 different protein-ligand complex conformations for each docked complex were generated through AUTODOCK suite of MGL Tools, the confirmation with lowest binding energy was displayed as the best binding energy.

Binding energy of the dataset ligands were shown in Table 5 along with the interaction amino acids and number of amino acids.

Table.No.5: EGFR inhibition of novel quinazoline-4-one derivatives II(3a-3j)-Glide dock scores of the dataset ligands.

Compound No	Binding Energy (Kcal/mol)	No of H-bonds	Interacting amino acids	H-bond lengths (Å)
II-3a	**-9.4**	2	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, LEU:820, ASP:831	2.98, 3.06
II-3c	**-9.6**	1	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, LEU:820, ASP:831	3.03
II-3d	-9.2	2	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, LEU:820, ASP:831	3.05, 2.95
II-3f	**-9.3**	2	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, MET:769, LEU:820, ASP:831	2.63, 2.54
II-3h	-9.1	1	LEU:694, VAL:702, LYS:721, ALA:719, LEU:820, ASP:831, GLY:772, LEU:764	2.75
II-3j	-9.1	1	LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, GLU:738, ASP:831, LEU:820	2.97

From these results, among the docked ligands, compounds **II-(3a-3j)** reported the binding energy ranged from -9.1 to -9.6 Kcal/mol and all compounds possess one/two hydrogen bonds each with LEU:694, PHE:699, VAL:702, LYS:721, ALA:719, LEU:820, ASP:831, MET:769, ASP:831 amino acids. Compounds **II-3c** & **II-3a** possess highest binding energy than all designed compounds.



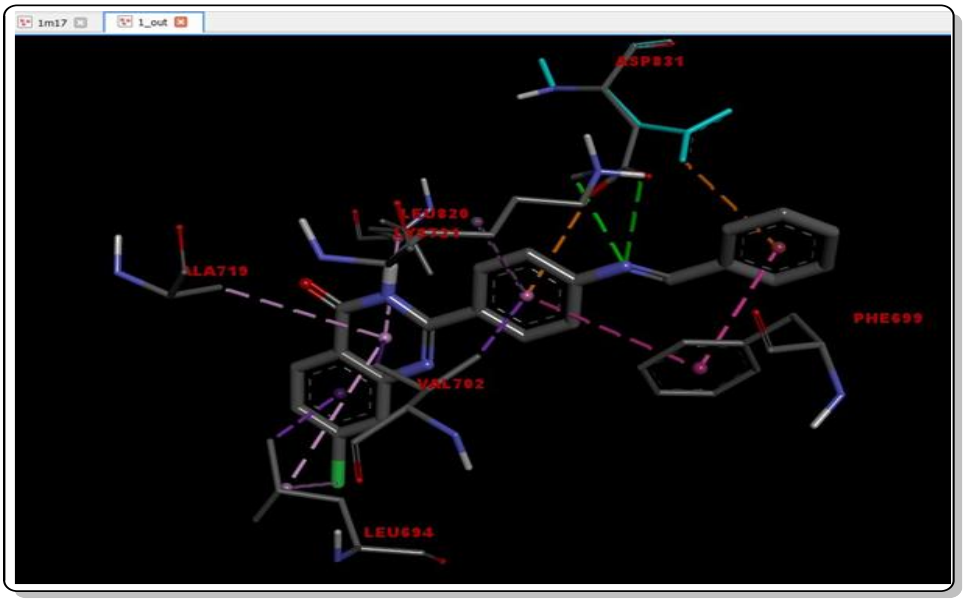
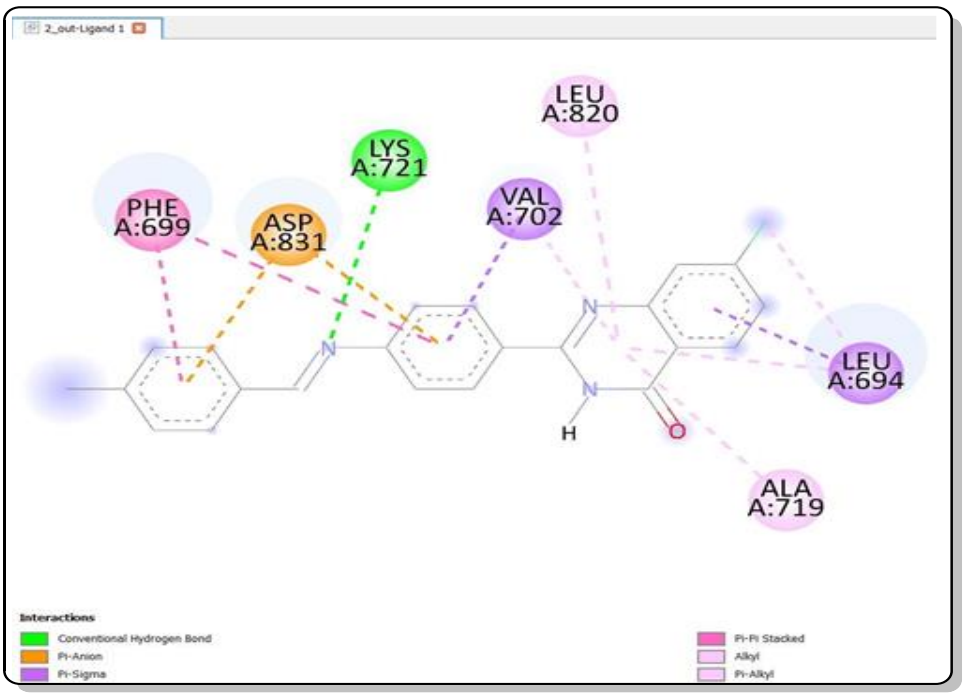


Figure.7. Docking Pose between the Ligand and the Protein (Dock1 and Dock-2)-Compound II-3a



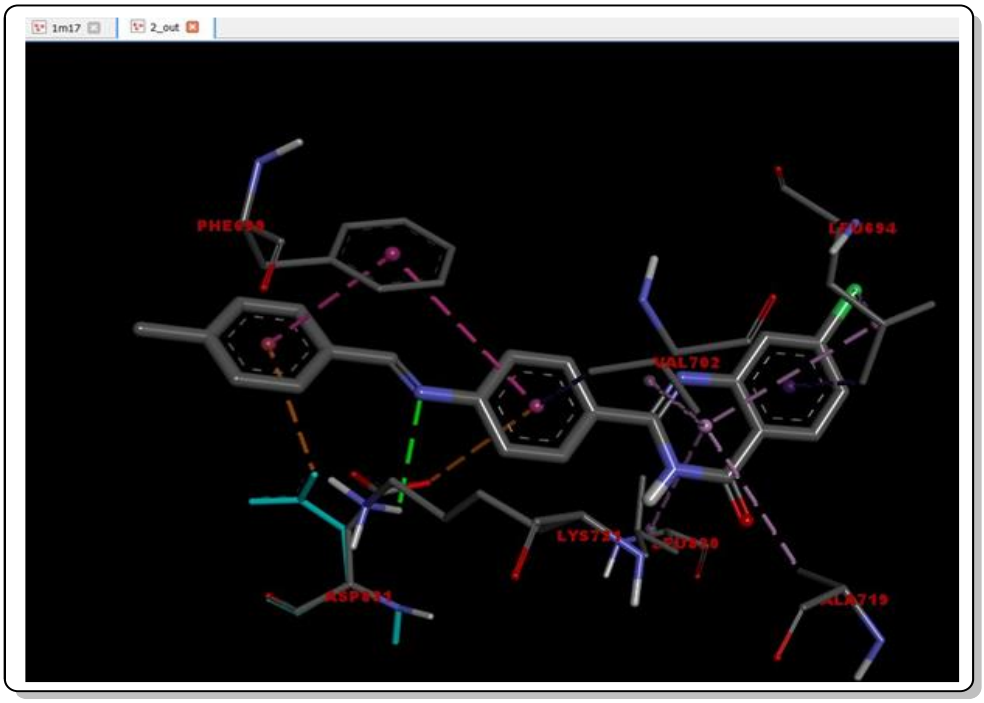


Figure.8. Docking Pose between the Ligand and the Protein (Dock1 and Dock-2)-Compound II-3c

4. CONCLUSION

In effort to find novel derivatives, a series of Quinazoline-4-one compounds was prepared and conformed. The yield of the synthesized compounds was found in the range of 74-88%. The present study showed that the antibacterial, anticancer and anthelmintic activities of compounds II-(3a-3j) may altered the introducing of a specific groups. That results, the quinajoline-4-one could be excellent and elegant to stimulate major advances in the cancer treatment of remarkable significance in pharmaceutical screening.

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REFERENCES

1. Zhang Z, Wang M, Zhang C, Zhang Z, Lua J, Wang F. The cascade synthesis of quinazolinones and quinazolines using an α-MnO2 catalyst and tert-butyl hydroperoxide (TBHP) as an oxidant. Chemical Communications. 2015; 51: 9205-9207. DOI: 10.1039/ C5CC02785C.
2. Guo S, Li Y, Li T, Zhang W, Fan X. ChemInform abstract: Rapid assembly of quinazolinone scaffold via copper-catalyzed tandem reaction of 2-bromobenzamides with aldehydes and aqueous ammonia: Application to the synthesis of the alkaloid tryptanthrin. RSC Advances. 2014; 4: 59289-59296. DOI: 10.1039/C4RA10799C.
3. Natte K, Neumann H, Wu X-F. Pd/C as an efficient heterogeneous catalyst for carbonylative four-component synthesis of 4(3H)-quinazolinones. Catalysis Science & Technology. 2015; 5: 4474- 4480. DOI: 10.1039/C5CY00907C.
4. Wdowiak P, Matysiak J, Kuszta P, Czarnek K, Niezabitowska E, Baj T. Quinazoline derivatives as potential therapeutic agents in urinary bladder cancer therapy. Front Chem. 2021. https://doi.org/10.3389/fchem.2021. 765552.
5. Dohle W, Jourdan FL, Menchon G, Prota AE, Foster PA, Mannion P, et al. Quinazolinone-based anticancer agents: synthesis, antiproliferative SAR, antitubulin activity, and tubulin co-crystal structure. J Med Chem. 2018; 61(3): 1031–44.
6. Syed T, Asiri YI, Shaheen S, Gangarapu K. Design, synthesis and anticancer evaluation of structurally modifed substituted aryl-quinazoline derivatives as anticancer agents. Synth Commun. 2021; 51(18): 2782–95.
7. Abou-Seri SM, Taha AM, Mohamed MA, Abdelkader NM. New quinazoline-sulfonylurea conjugates: design, synthesis and hypoglycemic activity. Med Chem. 2019; 15(6): 634–47.
8. Shalaby AA, El-Khamry AMA, Shiba S, Ahmed AAAEA, Hanaf AA. Synthesis and antifungal activity of some new quinazoline and benozoxazinone derivatives. Archiv der Pharmazie Int J Pharm Med Chem. 2000; 333(11): 365–72.
9. Hassanzadeh F, Jafari E, Hakimelahi G, Khajouei MR, Jalali M, Khodarahmi G. Antibacterial, antifungal and cytotoxic evaluation of some new quinazolinone derivatives. Res Pharm Sci. 2012; 7(2): 87.
10. Khodarahmi G, Khajouei MR, Hakimelahi G, Abedi D, Jafari E, Hassanzadeh F. Antibacterial, antifungal and cytotoxic evaluation of some new 2, 3-disubstituted 4 (3H)-quinazolinone derivatives. Res Pharm Sci. 2012; 7(3): 151.
11. Honkanen E, Pippuri A, Kairisalo P, Nore P, Karppanen H, Paakkari I. Synthesis and antihypertensive activity of some new quinazoline derivatives. J Med Chem. 1983; 26(10): 1433–8.
12. Dutta A, Sarma D. Recent advances in the synthesis of Quinazoline analogues as Anti-TB agents. Tuberculosis. 2020; 124: 10198.
13. Shao Z, Jahanbani A, Sheikholeslami SM. Multiplicative topological indices of molecular structure in anticancer drugs. Polycyclic Aromat Compd. 2022; 42(2):475–88.
14. Mahmoud HK, Gomha SM, Farghaly TA, Awad HM. Synthesis of thiazole linked imidazo [2, 1-b] thiazoles as anticancer agents. Polycyclic Aromat Compd. 2021; 41(8): 1608–22.
15. Auti PS, George G, Paul AT. Recent advances in the pharmacological diversifcation of quinazoline/quinazolinone hybrids. RSC Adv. 2020; 10(68): 41353–92.
16. El-Metwally SA, Abou-El-Regal MM, Eissa IH, Mehany AB, Mahdy HA, Elkady H, et al. Discovery of thieno [2, 3-d] pyrimidine-based derivatives as potent VEGFR-2 kinase inhibitors and anti-cancer agents. Bioorg Chem. 2021; 112: 10494
17. Wang, D.; Gao, F. Quinazoline derivatives: Synthesis and bioactivities. Chem. Cent. J. 2013, 7, 1–15.
18. X, Lin Q , Wang L. Onepot solvent-free synthesis of 2, 3-disubstituted 4(3H)-quinazolinones catalyzed by long-chain double SO_3H functionalized Brønsted acidic ionic liquids under microwave irradiation. Journal of the Iranian Chemical Society. 2015; 12: 897-901. DOI: 10.1007/ s13738-014-0553-0.
19. Sharif, M. Quinazolin-4(3H)-ones: A tangible synthesis protocol via an oxidative olefin bond cleavage using metal-catalyst free conditions. Appl. Sci. 2020; 10: 281-295.
20. Alanazi AM, Abdel-Aziz AAM, Shawer TZ, Ayyad RR, Al-Obaid AM, Al-Agamy Mohamed HM, Maarouf AR and El-Azab AS: Synthesis, antitumor and antimicrobial activity

of some new 6-methyl-3-phenyl-4(3H)-quinazolinone analogues. Journal of Enzyme Inhibition and Medicinal Chemistry 2015; 31: 721-35.

21. Kumar A, Sharma P, Kumari P and Kalal BL: Exploration of antimicrobial and antioxidant potential of newly synthesized 2, 3-disubstituted quinazoline-4(3H)-ones. Bioorganic and Medi Chem Letters 2011; 21: 4353-57.
22. Rasha SG and Mohsen MK: Synthesis and reactions of some new quinazoline derivatives for In-vitro evaluation as anticancer and antimicrobial agents. Journal of Heterocyclic Chemistry 2018; 10: 1-8.
23. Kouznetsov VV, Robles-Castellanos ML, Sojo F, RojasRuiz1 FA, Arvelo F. Diverse C-6 substituted 4-methyl-2-(2-, 3- and 4-pyridinyl) quinolines: synthesis, in vitro anticancer evaluation and in silico studies. Med Chem Res 2017; 26: 551-561.
24. Mehta S, Kumar S, Marwaha RK, Balasubramanian N, Kalavathy R, Siong Meng L, Ali Shah SA and Vasudevan M: Synthesis, molecular docking and biological potentials of new 2-(4-(2-chloroacetyl) piperazin-1-yl)- N-(2-(4- chlorophenyl)-4- oxoquinazolin-3(4H)-yl) acetamid derivatives. BMC Chemistry 2019; 13(113): 1-21.
25. Abuelizz HA, Hassane AE, Marzouk M, Ezzeldin E, Ali AA and Al-Salahi R: Molecular modeling, enzyme activity, anti-inflammatory and antiarthritic activities of newly synthesized quinazoline derivatives. Future Medicinal Chemistry 2017; 9(17): 1995-09.